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Influence of CLA addition in non-ruminant diets on lipid index values

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Abstract: In monogastric animals, tissue fatty acid profile directly reflects the fatty acid profile present in the animal's diet. Inadequate ratio of fatty acids in food can lead to negative effects on human health. Conjugated linoleic acid (CLA) is a group of isomers of linoleic acid (C18:2), and its most interesting role is in the prevention of tumors, atherosclerosis and diabetes. CLA is found in ruminant meat and milk, and since pigs and poultry do not have the ability to synthesize CLA, it is possible to add them to animal feed with biotechnological solutions. The scientific public imposes modern parameters for determining the nutritional value of fatty acids, in which the AI – index of atherogenicity, TI – index of thrombogenicity and H/H – hypocholesterolemic/hypercholesterolemic ratio are distinguished. The aim of this study was to determine the effect CLA addition to the diet of non-ruminants on the lipid indices of certain categories of meat, from the aspect of consumer health needs. A significant influence of the correction of feed's fatty acid composition on the lipid indices in food of animal origin was determined.

1. Introduction

The basic task of production animal feeding is to achieve the highest possible production of quality meat with minimal feed consumption and the lowest possible production costs. Animal nutrition has gone through several stages in its development, starting from complete empiricism to the modern concept of nutrition, which is based on very precise research of metabolic and biochemical changes at the cellular level. The goal of fattening pigs and broilers is to increase the body weight of the animal to obtain a larger amount of meat and fat by using mostly plant nutrients, with a smaller amount of nutrients of animal origin. With the development of scientific disciplines in the field of nutrition, as well as consumer awareness, the imperative in animal husbandry has become not only the quantity of food produced, but also the quality of food produced.

Fats are a heterogeneous group of compounds of different structures that are insoluble in water and soluble in organic solvents (ether and chloroform). The role of fat in human and animal nutrition is primarily energetic. Oxidation of one gram of fat yields 37 kJ, but in the case of carbohydrates, the yield is 17 kJ [2]. The raw materials for the synthesis of unsaturated fatty acids in the body are essential fatty acids that are ingested through feed. Essential fatty acids serve as the building units of many hormones, especially prostaglandins, leukotrienes, thromboxanes and others, but they are also a very important component of cell membranes. Fat requirements in monogastric animals are considered to be relatively small, up to 2%. Lack of essential fatty acids can lead to severe disorders. Up to 6% fat in the mixture accelerates the utilization of feed.



The chemical composition of food of animal origin, especially certain ingredients such as fatty acids, has attracted the attention of experts for years because of their impact on human health [4]. There are many examples in the literature where certain practices in nutrition and breeding increase the content of n-3 unsaturated and other desirable fatty acids in meat, milk and eggs. If a certain animal nutrition strategy is adopted, the results can be seen in a short period of time. Importantly, the change in the ratio of n-6/n-3 unsaturated fatty acids acid in the human diet has become a cause for concern, as this ratio has changed in favor of n-6 unsaturated fatty acids due to lifestyle and diet in which fish and vegetables are declining [15]. The fat content and fatty acid composition of meat has changed from that in grazing animals to that in intensively kept and fed animals in modern technological conditions. The n-6/n-3 ratio in the body is one of the main parameters for determining the nutritional value of fat, given the effects it has on the body.

The taste of meat is mostly determined by the fatty acid composition of intramuscular fat. Also, the taste of semi-shelf stable (chilled) and shelf-stable meat products largely depends on the composition of fat depots. The amount of fat in the carcass is mostly determined by genetic predispositions and the composition of feed and the fatty acid composition is determined by the fatty acids in the diets of monogastric animals. This is explained by the ability of pigs and broilers to absorb a large percentage of fat from feed in unchanged form [8]. This effect depends on the amount and duration fats are consumed. In fattening, by using dietary supplements, more nutritionally valuable food can be produced if the source of fatty acids is taken into account. Conjugated linoleic acid (CLA) is a term used for a group of isomers of linoleic acid (C18: 2), which has been proven to improve the quality of fat, so its biological role is assured. CLA have always been part of the human diet, and they are found in ruminant meat and milk [3], but not in products from monogastric animals. Primarily, CLA has the role of a micronutrient, and its most interesting role is in the prevention of tumors, atherosclerosis and diabetes [10].

These facts have led to new challenges for experts who care about animal nutrition, and thus indirectly about human health. With biotechnological solutions, it is possible to add CLA to feed for non-ruminants, since pigs and poultry are not able to synthesize this group of fatty acids. The quantitative ratio of fatty acids as biological components in the diet plays an important role in maintaining human health. As a consequence, modern parameters for determining the nutritional value of fatty acids are the index of atherogenicity (AI), index of thrombogenicity (TI) and hypocholesterolemic/hypercholesterolemic ratio (H/H). The AI and TI were developed by Ulbricht and Southgate in 1991 [17]. H/H was first proposed by Santos-Silva et al. in 2002 [14]. The H/H ratio could serve to protect consumers from hypercholesterolemia, but has some limitations. Similar to the AI and TI, the H/H ratio might include more kinds of fatty acids such as other molecular species of monounsaturated fatty acids (MUFA), and different weights can be assigned to different molecular fatty acid species [6]. Proper calculation of the AI and TI determines the potential for cardiovascular diseases in humans who consume meat. The aim of this study was to determine the effect of CLA addition in the diet of pigs and broilers on these lipid indices in meats from the animals.

2. Materials and Methods

Broilers of Cobb 500 provenance (60) with an initial average body weight of 40 g were used. Broilers were divided into two experimental groups (Group C – control group and Group E – experimental group) of 30 individuals each and fed with complete feed mixture for broilers of standard raw material and chemical composition (Table 1). Three mixtures were used, complete mixture for fattening broilers I (starter), complete mixture for fattening broilers II (grower) and complete mixture for fattening broilers III (finisher), which completely met the needs of broilers [11]. The groups differed, so for the experimental group, 2% CLA Lutalin® from BASF was added to the diets at all stages of fattening. Total CLA content in the

complete mixture for broilers in group E after the addition of the preparation was $4.43 \pm 0.15\%$. CLA was not detected in the complete mixture for broilers in group C.

Pigs (40) were from the mother of a crossbreed of Yorkshire and Landrace and the father of a Duroc, and had initial body weight of 60 kg. Pigs were divided into two experimental groups (C group – control group and E group – experimental group) of 20 individuals each and fed with a complete mixture for feeding pigs of standard raw material and chemical composition (Table 1). The complete mixture for fattening of pigs (finisher) completely met the needs of pigs [12]. The groups differed, so 2% CLA Lutalin® from BASF was added to the diet of the experimental group. The total CLA content in complete mixture for the pigs in group E was $5.12 \pm 0.03\%$ (individually). CLA was not detected in the complete mixture for pigs in group C.

The Lutalin® preparation used, manufactured by BASF, is an oil with an energy value of 9 kcal/g, produced by chemical isomerization from sunflower oil in the form of CLA methyl esters. Lutalin® contains CLA *trans*-10,*cis*-12 and *trans*-9,*cis*-10 isomers in a 1:1 ratio. At the end of fattening, six individuals from each group of animals were sacrificed in both experiments and samples of breast and drumstick meat were taken for analysis in broilers, while muscle tissue and smoked pork neck (after processing) was examined in pigs.

Chemical analyses to determine protein, moisture, cellulose, fat, and ash of the feed were conducted according to AOAC methods [1].

Table 1. Raw material and chemical composition of complete mixtures for broilers in fattening (group C and group E), (%)

Raw material composition of the mixture						
Component	Groups					
	Starter C	Starter E	Grower C	Grower E	Finisher C	Finisher E
Corn	50.85	48.85	44.15	42.15	44.95	42.95
Wheat	-	-	10.00	10.00	15.00	15.00
Full fat soya	15.00	15.00	17.00	17.00	20.00	20.00
Soybean meal	12.40	12.40	1.00	1.00	1.00	1.00
Soybean cake	17.00	17.00	23.30	23.30	14.70	14.70
Monocalcium phosphate	1.20	1.20	1.00	1.00	0.90	0.90
Chalk	1.60	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Premix	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Adsorbent	0.20	0.20	0.20	0.20	0.20	0.20
CLA	-	2	-	2	-	2
Chemical composition of the mixture						
Mixture	Groups	Protein	Moisture	Lipid	Ash	Cellulose
		X ± SD				
Complete mixture for	C	24.98±0.57	8.04±0.24	6.09±0.37	5.45±0.14	2.04±0.05
	E	24.97±0.47	8.06±0.27	6.96±0.35	5.50±0.15	2.04±0.04

broilers I (starter)						
Complete mixture for broilers II (grower)	C	22.17±0.21	9.38±0.09	7.03±0.26	4.88±0.13	2.16±0.04
	E	22.11±0.47	9.38±0.10	7.09±0.29	4.92±0.12	2.16±0.05
Complete mixture for broilers III (finisher)	C	20.91±0.87	9.98±0.07	5.44±0.11	4.76±0.21	2.38±0.26
	E	20.78±0.80	10.00±0.06	5.46±0.06	4.72±0.22	2.57±0.24

Table 2. Raw material and chemical composition of complete mixture for fattening pigs (group C and group E), (%)

Raw material composition of the mixture						
Komponenta	Finisher C		Finisher E			
Corn	48		46			
Barley	28		28			
Soybean meal	16		16			
Wheat bran	5		5			
Troumix 210	3		3			
Lutalin CLA	0		2			
Total	100		100			
Chemical composition of the mixture						
Mixture	Groups	Protein	Moisture	Lipid	Ash	Cellulose
		X ± SD				
Complete mixture for pig nutrition III (finisher)	C	15.22	12.28	2.96	2.68	4.17
	E	15.22	12.28	2.96	2.68	4.17

After sacrificing animals, the fatty acid composition of breasts and thighs with drumsticks of broilers and muscle tissue and smoked pork neck of pigs (n = 6) was determined and on the basis of the fatty acid composition, lipid indices (AI, TI, H/H) were calculated.

The fatty acids in meat and meat products were determined according to Milanković et al [9]. The fatty acid content is expressed as a percentage (%) of the total fatty acids identified.

The calculations of lipid indices were according to the following formulae:

$$AI = [(C12:0) + (4 \times C14:0) + (C16:0)] / [\Sigma n6 + \Sigma n3 + \Sigma MUFA]$$

$$TI = [(C14:0) + (C16:0) + (C18:0)] / [0.5 \times \Sigma MUFA + (0.5 \times \Sigma n6) + (3 \times \Sigma n3) + (\Sigma n3 / \Sigma n6)]$$

$$H/H = [(C18:1 + C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:4 + C22:5 + C22:6) / (C14:0 + C16:0)]$$

Statistical data processing was done in GraphPad Prism software version 7.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). The results are presented graphically as intermediate values using Microsoft Office Excel 2010. Two-way ANOVA with Tukey's multiple comparison test was performed to compare lipid indices among examined groups of broilers and pigs. Statistical significance is shown at $P < 0.05$.

3. Results and Discussion

Table 3 shows the values of AI, TI and H/H of breast and drumsticks with thighs of control and experimental groups of broilers.

Table 3. Lipid indices of control and experimental group of breasts and drumsticks with thigh of broilers (n = 6)

	Experimental group				P-value		
	Breast		Drumstick with thigh		Diet (row factor)	Meat (column factor)	Interaction (RxC)
	Control group	Experimental group	Control group	Experimental group			
AI	0.387 ^a	0.467 ^c	0.303 ^b	0.473 ^c	*	*	*
TI	2.732	2.637	2.875	2.673	ns	ns	ns
H/H	2.675 ^a	2.300 ^b	2.750 ^a	2.247 ^{ac}	*	ns	*

Legend: Within a row, means with a different superscript letter significantly differ (^{a,b,c,d} – $P < 0.05$); ns = no significance ($P > 0.05$); * ($P < 0.05$).

From the presented results, the AI of drumstick with thigh of the control group broilers was significantly lower than the AI of the control group breasts ($P < 0.05$). The AI of the breast and drumstick with thighs of the experimental group of broilers was significantly higher than the AI of the breast and drumstick with thighs of the control broilers ($P < 0.05$). No significant difference was found between the AIs of the breast and the drumstick with thigh in the experimental group of broilers. The diet had a significant ($P < 0.05$) effect on AIs, and a significant difference was observed between different types of meat.

No significant differences were found between the TIs of the breast and drumstick with thigh of the control and experimental groups of broilers, as well as their mutual interactions.

Broilers fed with added CLA had a statistically significantly lower H/H indices in both breast meat and drumsticks with thighs compared to the H/H indices of these two types of meat of the broiler control group ($P < 0.05$). A significant interaction between diet and meat type was found ($P < 0.05$).

Table 4 shows the AI, TI and H/H of muscle tissue and smoked pork neck of the control and experimental groups of pigs.

Table 4. Lipid indices of control and experimental group of muscle tissue and smoked pork neck of pigs (n = 6)

	Experimental group				P-value		
	Muscle		Smoked pork neck				
	Control group	Experimental group	Control group	Experimental group	Diet (row factor)	Meat (column factor)	Interaction (RxC)
AI	0.552 ^a	0.895 ^c	0.573 ^b	0.853 ^d	*	ns	*
TI	1.465 ^a	2.195 ^c	1.530 ^b	2.048 ^d	*	*	*
H/H	0.593 ^a	0.350 ^c	1.962 ^b	1.370 ^d	*	*	*

Legend: Within a row, means with a different superscript letter significantly differ (^{a,b,c,d} - $P < 0.05$); ns = no significance ($P > 0.05$); * ($P < 0.05$).

From the presented results, the AIs of muscle tissue and smoked pork neck of the experimental group of pigs was significantly higher than the AIs of muscle tissue and smoked pork neck of the control group of pigs ($P < 0.05$). The AI of muscle tissue of the experimental group of pigs was significantly higher than the AI of smoked pork neck of the experimental group of pigs ($P < 0.05$), while the AI of muscle tissue of the control group of pigs was significantly lower than the AI of smoked pork neck of the control group of pigs ($P < 0.05$). A statistically significant interaction ($P < 0.05$) was found between the type of meat and the diet of pigs.

The TIs of muscle tissue and smoked pork neck of the control group of pigs were significantly lower than the TIs of the examined types of meat of the experimental group of pigs ($P < 0.05$). Significant differences were found between the TIs of both muscle tissue and smoked pork neck of the control and experimental groups of pigs ($P < 0.05$). The interaction between diet and meat type on TI was statistically significant ($P < 0.05$).

The experimental group of pigs had significantly lower H/H indices of muscle tissue and smoked pork neck than the H/H indices of the examined types of meat of the control group of pigs ($P < 0.05$). A statistically significant interaction of the H/H index ($P < 0.05$) was found between the compared parameters.

Among the risk factors for the development of chronic non-infectious diseases (cardiovascular and cerebral diseases, arterial hypertension, malignant neoplasms, diabetes, obesity, biliary tract calculosis, osteoporosis, dental caries), nutrition is of great importance. Today, the scientific community knows that the chemical and fatty acid composition of meat depends on the composition of animal feed, which has an indirect impact on human health. The negative attitude towards meat consumption has a number of different causes. One of the reasons is the debatable nutritional value of meat if we take into account the values of lipid indices (AI, TI and H/H) which have a direct impact on the risk of disease in humans. Lower AI and TI indicate more nutritionally valuable food, so they help prevent cardiovascular diseases related to fat intake [17]. A higher H/H index indicates a higher nutritional value of fat. The addition of CLA to animal feed, as one of the strategies in changing the fatty acid ratio of meat, indirectly enables the management of AI, TI and H/H indices.

In studies with broilers fed with mixtures with different CLA concentrations (0.2%, 3%), Du and Ahn found that the average content of total saturated fatty acids increased significantly as the CLA concentration in broiler feed mixtures increased, while the average content of total monounsaturated and polyunsaturated

fatty acids decreased [7], which has negative consequences on the AI and TI. Sirri et al. observed that in broilers fed diets with different concentrations of CLA (0; 2%; 4%) the content of n-3 fatty acids in the meat of broiler drumstick with thigh decreased as the concentration of CLA in the feed mixture increased [16], which is not in accordance with the our results for TI in broilers.

According to Cech et al., statistically significant differences in the n-3/n-6 ratio as well as in the content of n-3 and n-6 fatty acids occurred in pigs fed with 2% CLA, which ultimately meant a decrease in the TI index, and so there was a positive impact on disease prevention in humans [5]. The results so far confirm that the use of CLA in the diet of pigs affects the fatty acid composition of meat [13], i.e., it increases the saturated fatty acid content in intramuscular fat, and reduces monounsaturated fatty acids. These data are in compliance with the results of this study in pigs, because through the AI, TI and H/H achieved, the interaction of CLA in animal diet in both types of meat was proven.

4. Conclusion

The addition of 2% CLA, correcting the fatty acid composition of animal feed, significantly affects the AI, TI and H/H indices of pig meat and the AI and H/H indices of broiler meat, while the TI of broiler meat is not affected.

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